Vector Relations

Detection of Tomato Spotted Wilt Virus in Individual Thrips
by Enzyme-Linked Immunosorbent Assay

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) was used to detect tomato spotted wilt virus (TSWV) in individual thrips. TSWV was readily detected in 210 of 391 *Frankliniella occidentalis* and 24 of 120 *F. schultzei* laboratory-grown adult thrips that were provided acquisition access on infected host plants as larvae. In transmission tests, higher rates of TSWV-transmission occurred with three as opposed to one insect. With three insects, six positive transmissions occurred out of seven (86%) ELISA-positive *F. occidentalis* groups, and seven occurred out of eight (88%) ELISA-positive *F. schultzei* groups. In single transmission tests, 14 positive transmissions occurred out of 186 (7%) ELISA-positive *F. occidentalis* and none occurred out of two ELISA-positive *F. schultzei*. TSWV was detected in 32 of 275 (12%) adult and 233 of 527 (44%) larval thrips removed from TSWV-infected lettuce plants obtained from three Hawaii farms.

Tomato spotted wilt virus (TSWV) is unique because it has one of the widest known host ranges of any plant virus; it is the only virus transmitted in a persistent manner by certain species of thrips; it is highly unstable in vitro; and it is covered by a lipoprotein envelope (4). TSWV causes serious diseases in a number of economically important crops worldwide. Recently, several published and informal reports indicate that this disease is becoming an important problem in several states in the United States and Canada. Crops affected include ornamentals grown on the east (1) and west coasts (2), and tomato, pepper, tobacco (16), and peanuts in several southern and southeastern states. In

Hawaii, TSWV accounts for major crop losses. It affects the production of lettuce, tomato, bell pepper, and chrysanthemum. The western flower thrips, *Frankliniella occidentalis* (Pergande), is the most important TSWV vector in Hawaii's vegetable-growing regions (10,11).

Previous studies (10,11) demonstrated a significant correlation between western flower thrips numbers and TSWV disease incidence in a Hawaii lettuce farm. A simple means for detection of TSWV in vector thrips would be useful in predicting potential disease outbreaks. The enzyme-linked immunosorbent assay (ELISA) has been used to detect plant viruses in individual aphids (12–14) and planthoppers (7,18). Previously we reported the use of ELISA in studies for TSWV detection in reservoir weed hosts (8). Here we report the first use of ELISA to detect TSWV in

individual thrips obtained from laboratory-reared colonies and from the field and compare those results with infectivity tests. A preliminary report of this work has been published (9).

MATERIALS AND METHODS

Enzyme-linked immunosorbent assay. The double antibody sandwich ELISA method was used to detect TSWV in individual thrips. The antiserum was produced from a TSWV strain obtained from an infected lettuce plant on the island of Maui (15). We have used this polyclonal antiserum extensively to detect TSWV using ELISA in reservoir weed hosts of TSWV in Hawaii's vegetable-growing regions, and to confirm TSWV infection of inoculated greenhouse-grown plants that included several weed species and papaya (*Carica papaya* L.) (8,15). Individual thrips were identified under a dissecting microscope at ×100 magnification and removed from storage vials with a fine-tipped camel's hair brush, placed into separate wells of a polyvinyl chloride microtiter plate (Dynatech, Alexandria, VA), triturated with the blunt end of a small glass rod and 50 μl of ELISA extraction buffer (0.01 M sodium-potassium phosphate buffer, pH 7.4, containing 0.02% sodium azide [w/v], 0.8% sodium chloride [w/v], 0.05% Tween 20 [v/v]) and 2% polyvinylpyrrolidone, mol wt 40,000 ([w/v] Sigma Chemical Co., St. Louis, MO) added per well. The suspension was transferred to U-bottom Immulon 2 microelisa plates (Dynatech, Alexandria, VA) previously coated with 200 μl of TSWV immunoglobulin (1 μg/ml) and incubated overnight at 4 C. Five to six wells were filled with ELISA extraction buffer without insect extract. TSWV-infected *Nicotiana benthamiana* Domin tissue sap 1/50 (w/v) prepared in ELISA extraction buffer was added to other wells.

Alkaline phosphatase-conjugated immunoglobulins were cross absorbed with healthy *N. benthamiana* tissue extract (1:20, w/v) for 15 min at room temperature (25–27 C), added at 1/2,000 dilution, and incubated for 4 to 5 hr at 30 C. Plates were washed three times between each step with phosphate-buffered saline containing 0.05% Tween 20. *p*-Nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) was added at 1 mg/ml, incubated for 1 hr at room temperature. The reaction was terminated with the addition of 3 M NaOH. ELISA reactions were measured spectrophotometrically at 405 nm using an EL307B EIA reader (Bio-Tek Instruments, Winooski, VT), and positive readings were confirmed by visual observations. The A_{405nm} values presented were obtained by subtracting the buffer control absorbance values (average of three wells) from sample values. A sample was considered TSWV-positive if the A_{405nm} values were greater than twice the mean healthy thrips control values and exhibited a visible yellow color not observed in control wells. Minimum positive A_{405nm} value was 0.085.

Thrips. Insects used in our studies were obtained from laboratory-reared colonies of thrips and from lettuce plants (*Lactuca sativa* L.) removed from three Hawaii farms. A fine-tipped brush (size 000 erminette) was used in collecting, transferring, and removal of thrips from plants.

Virus-free colonies of *F. occidentalis* and *F. schultzei* Trybom (pale form) were maintained in the laboratory on bean pods (*Phaseolus vulgaris* L. 'Green Crop'). Colonies were started from nymphs that emerged from eggs oviposited on bean pods by *F. occidentalis* adults collected from *Chrysanthemum morifolium* (Ram.) Hemsl. and *F. schultzei* adults collected from *Leucaena glauca* L. blossoms obtained from the field. Both thrips colonies were maintained at 26 C with a 10-hr light and 14-hr dark cycle.

Thrips were placed in small vials containing 50 μl of ELISA extraction buffer and stored at 4 C until analyzed for TSWV by ELISA.

Effects of storage of thrips in buffer on TSWV-detection. Preliminary studies examined TSWV detection in thrips after storage time in ELISA extraction buffer at 4 C. Laboratory-reared *F. occidentalis* first instar larvae and *F. schultzei* adults that were given access to TSWV-infected *Emilia sonchifolia* (L.) DC. plants for 3 days were placed in buffer and examined immediately (0), 1, 3, 6, 12, and 24 days after removal from plants. Larval and adult thrips fed on healthy plants served as controls. Fifty thrips per

treatment were analyzed by ELISA at each sample time. A Student's *t*-test for paired comparisons (19) was used to analyze differences in A_{405nm} values between thrips fed on TSWV-infected and healthy plants.

Larval and adult thrips were collected from TSWV-infected lettuce plants at three farms located on the island of Oahu. A total of 10 plants per farm were randomly selected, placed separately in individual plastic bags, and brought back to the laboratory. Thrips were removed by immersing and agitating individual plants in 1% detergent (Liquinox) for 2–3 min, and the thrips separated by pouring the mixture through nested sieves (0.85 and 0.18 mm diameter openings). Thrips and debris retained on the lower sieve were washed in a petri dish with 70% ethanol. Thrips were identified under a dissecting microscope at ×100 magnification. *F. occidentalis* adults were transferred to 1-dram vials containing 3 ml of ELISA extraction buffer and stored at 4 C until assayed for TSWV using ELISA. Assays were performed within 6 days after thrips removal from plants.

Transmission tests. Six separate tests were conducted. Laboratory-reared *F. schultzei* and *F. occidentalis* first instar larvae were caged on the upper leaf surfaces of virus source plants for 3 days of acquisition access feeding. Separate groups of first instar thrips were also caged on virus-free plants as a control treatment. Source plants were TSWV-infected *E. sonchifolia* plants. Thrips were transferred and confined on TSWV-free bean leaves by modified Tashiro cages (24) to complete their development. The thrips completed the second larval instar, prepupal, and pupal development in these cages. Three days after adult emergence, thrips were removed and placed on lettuce seedlings (*L. sativa* var. *longifolia* Lam. 'Parris Island Cos'), in the four- to five-leaf stage of growth. In tests 1 and 2, three adult thrips were caged together as a source of TSWV. In tests 3, 4, 5, and 6, single adult thrips were caged on separate host plants.

After 3 days of inoculation access feeding, all thrips were removed, killed by immersion in 95% ethanol, and stored in vials containing ELISA extraction buffer. Plants were placed in a greenhouse and treated immediately and at 3- to 4-day intervals with abamectin (Avid 0.15 EC) at 12 μg a.i./ml to eliminate the development of larvae from eggs oviposited by thrips. Plants were kept for 30 days and observed for TSWV symptom development. TSWV infection was confirmed by ELISA and mechanical inoculation on 1.5-mo-old Parris Island Cos seedlings.

RESULTS

Effect of storage on TSWV detection in thrips. Acquisition of TSWV by thrips and its retention in thrips stored in buffer was tested by assaying 50 individual thrips at different intervals. TSWV was readily detected by ELISA in larvae of *F. occidentalis* and adults of *F. schultzei* thrips that had been caged on infected plants for acquisition access feeding.

Mean A_{405nm} values ± standard deviations for TSWV-positive *F. occidentalis* second instar larvae were 0.49 ± 0.34 after 0 day, 0.32 ± 0.25 after 1 day, 0.47 ± 0.39 after 3 days, 0.32 ± 0.28 after 6 days, 0.28 ± 0.26 after 12 days, and 0.13 ± 0.02 after 23 days storage. In comparison, these values were significantly higher ($P < 0.001$) than A_{405nm} values obtained for thrips denied access to TSWV, which ranged from 0.01 to 0.02. TSWV was detected in 86% of the thrips that were tested immediately after acquisition access feeding (0 day), in 56% of thrips sampled after 1 day of storage in buffer, 58% after 3 days, 42% after 6 days, 28% after 12 days, and 42% after 23 days.

Mean A_{405nm} values ± standard deviations for *F. schultzei* adults sampled immediately after acquisition access feeding were 0.18 ± 0.1 (0 day), 0.15 ± 0.06 after 1 day of storage in buffer, 0.21 ± 0.16 after 3 days, 0.14 ± 0.05 after 6 days, 0.22 ± 0.11 after 12 days, and 0.10 ± 0.01 after 24 days of storage. These values were significantly higher ($P < 0.01$) than those of control thrips that ranged from 0.01 to 0.03. TSWV was detected in all of the thrips sampled after 0 day, in 80% of thrips sampled after 1 day of storage in buffer, 98% after 3 days, 88% after 6 days, 70% after 12 days, and 16% after 24 days of storage. Based on this information, all subsequent testing of

thrips was completed within 6 days after storage in buffer.

Detection of TSWV in individual thrips obtained from the field. TSWV was readily detected by ELISA in individual thrips removed from TSWV-infected field grown lettuce obtained from three farms located on the island of Oahu.

Seven of 81 adult and 69 of 175 larval thrips collected from 10 TSWV-infected lettuce plants from Farm 1 were ELISA-positive (Table 1). Mean percentage of TSWV-positive thrips per infected lettuce plant was 7% for adults and 36% for larvae. On Farm 2, seven of 48 adult and 80 of 187 larval thrips were ELISA-positive. The mean percentage of TSWV-positive thrips per infected lettuce plant was 19% for adults and 44% for larvae. On Farm 3, 18 of 146 adult and 84 of 165 larval thrips were ELISA-positive. The mean percentage of TSWV-positive thrips per infected lettuce plant was 12% for adults and 52% for larvae. Mean A_{405nm} values ± standard deviations for positive individuals ranged from 0.22 ± 0.16 to 0.42 ± 0.42 and were significantly higher than *F. occidentalis* individuals denied access to TSWV. All adult thrips assayed from all three farms were identified as *F. occidentalis*. Therefore, the majority of the larvae assayed were presumed to be *F. occidentalis*.

All lettuce plants from which thrips were obtained in this study were confirmed to be TSWV-positive via ELISA.

Relationship between TSWV detection and infectivity. Thrips previously placed on TSWV-infected plants for acquisition access feeding and on Parris Island Cos plants for inoculative feeding were assayed to compare TSWV-detection by ELISA and infectivity. TSWV was readily detected by ELISA in 210 of 391 *F.*

occidentalis and 24 of 120 *F. schultzei* late-stage adult thrips previously fed on TSWV-infected acquisition host plants as larvae (Table 2). All thrips fed on healthy host plants were ELISA negative. A_{405nm} values were variable and ranged from 0.09 to 1.89 for TSWV-positive thrips. A_{405nm} values for thrips fed on healthy plants were lower, ranging from 0 to 0.08.

In transmission tests, higher rates of TSWV-transmission were observed when more than one vector individual was used (Fig. 1). When three thrips were allowed inoculative feeding on lettuce, positive TSWV-transmissions were associated with ELISA-positive assays in seven out of eight (88%) cases with *F. schultzei* and in six out of seven (86%) cases with *F. occidentalis*. In the two cases where no association was observed, two thrips each apparently escaped during inoculative feeding and therefore were not ELISA assayed. When single insects were allowed inoculative feeding on lettuce, positive TSWV-transmissions were associated with ELISA-positive assays in 14 out of 186 (7%) cases with *F. occidentalis* and 0 out of two cases with *F. schultzei*. Mean A_{405nm} values for transmitter thrips (Table 2) were also higher in tests where multiple as opposed to single thrips were used in transmissions. No TSWV-transmissions occurred in any of the controls used in all tests.

DISCUSSION

Serological tests have been used to detect TSWV in thrips. Paliwal (20) detected TSWV by microprecipitation in extracts of

TABLE 1. Number of individual *Frankliniella occidentalis* thrips testing positive for tomato spotted wilt virus (TSWV) by double antibody sandwich enzyme-linked immunosorbent assay (ELISA), absorbance readings, and mean percentage of individual thrips TSWV-positive per TSWV-infected lettuce plant[x]

Farm	Thrips	(No.) thrips TSWV-positive/ total tested from 10 lettuce plants	ELISA A_{405nm} values[y] range (No.)	mean (±SD)[z]	Mean percentage TSWV-positive thrips/plant (±SD)[z]
1	adult	7/81	0.13–0.52	0.31 ± 0.16	7 ± 8
1	larva	69/175	0.09–1.04	0.29 ± 0.21	36 ± 24
2	adult	7/48	0.13–1.32	0.42 ± 0.42	19 ± 25
2	larva	80/187	0.09–1.41	0.35 ± 0.27	44 ± 22
3	adult	18/146	0.09–1.21	0.28 ± 0.32	12 ± 9
3	larva	84/165	0.09–0.80	0.22 ± 0.16	52 ± 14
Control	adult	0/27	0–0.01	0.004 ± 0.002	···
Control	larva	0/51	0–0.05	0.01 ± 0.02	···

[x] Ten individual lettuce plants selected randomly were collected from each of three Hawaiian farms.
[y] Values from thrips include only ELISA-positive values. Positive A_{405nm} values had a minimum value of 0.085 and exhibited a visible yellow color.
[z] SD = standard deviation.

TABLE 2. Number of individual *Frankliniella occidentalis* and *F. schultzei* adult thrips testing positive for tomato spotted wilt virus (TSWV) by double antibody sandwich enzyme-linked immunosorbent assay (ELISA), number of TSWV transmissions, ELISA absorbance readings of ELISA-positive and transmitter-positive thrips

Test no.	Plant condition	Thrips species	TSWV ELISA-positive thrips Thrips/ total tested (no.)	A_{405nm} value mean (± SD)[x]	range	Positive transmission/ total tested (no.)	ELISA A_{405nm} values of transmitter thrips[w] mean (± SD)[x]	range
1	Infected	*F. schultzei*[y]	23/48	0.80 ± 0.57	0.09–1.88	8/21	0.32 ± 0.38	0–0.89
1	Healthy	*F. schultzei*[y]	0/29	0.002 ± 0.01	0–0.02	0/13	···	···
2	Infected	*F. occidentalis*[y]	24/41	0.58 ± 0.48	0.09–1.89	7/19	0.63 ± 0.64	0.01±1.88
2	Healthy	*F. occidentalis*[y]	0/25	0.003 ± 0.02	0–0.06	0/13	···	···
3	Infected	*F. occidentalis*[z]	10/49	0.23 ± 0.12	0.11–0.46	5/49	0.15 ± 0.18	0.01–0.46
3	Healthy	*F. occidentalis*[z]	0/32	0.002 ± 0.004	0–0.01	0/32	···	···
4	Infected	*F. schultzei*[z]	1/72	0.32	0.09–0.32	0/72	0.01	···
4	Healthy	*F. schultzei*[z]	0/98	0.02 ± 0.01	0–0.06	0/98	···	···
5	Infected	*F. occidentalis*[z]	64/104	0.28 ± 0.22	0.09–0.89	4/104	0.37 ± 0.32	0.11–0.83
5	Healthy	*F. occidentalis*[z]	0/114	0.02 ± 0.01	0–0.08	0/114	···	···
6	Infected	*F. occidentalis*[z]	112/197	0.32 ± 0.26	0.09–1.55	5/197	0.11 ± 0.16	0.01–0.35
6	Healthy	*F. occidentalis*[z]	0/74	0.02 ± 0.01	0.004–0.07	0/74	···	···

[w] Values for inoculative thrips include only ELISA-positive values. Minimum ELISA-positive value was based on a value of 0.085. Values shown are adjusted against mean buffer values.
[x] SD = standard deviation.
[y] Three thrips used in transmission tests.
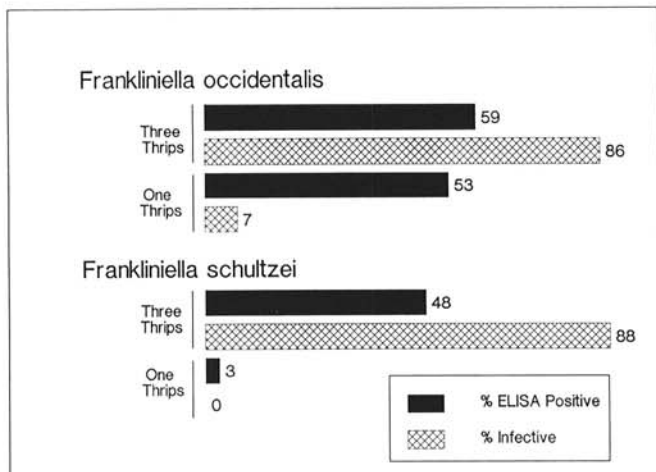[z] Single thrips used in transmission tests.

Fig. 1. Comparison of percentage of *Frankliniella occidentalis* and *F. schultzei* testing positive for tomato spotted wilt virus (TSWV) by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) and percentage of ELISA-positive thrips infective for TSWV as determined by tests of thrips individually or in groups of three.

50 *F. fusca* (Hinds) thrips fed on infected plants. Amin et al (3) detected TSWV in extracts of 11 *F. schultzei* adults and 20 *Scirtothrips dorsalis* Hood by the hemagglutination test. This study reports the first successful detection of TSWV in individual thrips.

TSWV was readily detected by ELISA in individual thrips fed on TSWV-infected plants in the laboratory and in thrips removed from TSWV-infected field-grown lettuce. ELISA values of virus-free vector extracts were substantially lower than virus-containing individuals and this validated the test.

The high A_{405nm} values obtained from individual thrips indicates that ELISA is quite sensitive for TSWV detection. ELISA offers the possibility of quantifying virus content and determining its distribution within thrips. These properties have been demonstrated with some aphid vectors of plant viruses (12,13).

Our results showed little correlation between detection of TSWV in individual thrips by ELISA and the ability of thrips to transmit the virus. The majority of thrips that were TSWV-positive by ELISA did not transmit the virus. Other investigators working on planthopper and aphid vector/virus systems have reported similar results (7,18). These results may be attributed to the complexities of insect vector/virus relationships. Transmission of persistent viruses, such as TSWV, can only occur after the virus invades the insect's salivary gland. Virus in some ELISA-positive individuals may not yet have penetrated the gut and moved to the salivary gland. In addition, infective virus levels in the salivary gland of an individual insect may be too low for effective transmission to occur. Aphid efficiency in transmitting pea enation mosaic virus and barley yellow dwarf virus has been reported to be dosage related (5,6,14,23), although this concept is currently controversial (22). Our results demonstrating that higher transmission rates occur with several thrips per test plant as opposed to single thrips suggest that the amount of TSWV transmitted may be important or that increased thrips numbers may increase the probability of satisfying critical events required for effective inoculation. For example, virus inoculation may only occur when the insect salivates into the appropriate plant cell. TSWV-positive thrips may not have fed efficiently on the test plants, thus reducing effective transmission.

We found Parris Island Cos particularly difficult to mechanically inoculate and infect with TSWV. This suggests that this cultivar may not be the most suitable host for transmission studies. Further studies have been initiated to determine if the low transmission rates observed in this study may be related to host susceptibility to virus or poor suitability for thrips.

In our studies, TSWV was readily detected in adult *F. schultzei* thrips that were previously fed on a TSWV-infected host plant.

This demonstrates that adults are capable of obtaining TSWV even though previous work by others (21) has shown they are incapable of transmitting. This would support, but not prove, the hypothesis that decreased gut permeability to virus occurs with increased age of the insect (17).

Our results clearly demonstrate that ELISA can be used to determine relative numbers of viruliferous thrips in relationship to time of year and farm location. In addition, the technique we have developed may be used to address questions relating to TSWV acquisition, persistence, and replication in thrips. When used in conjunction with reciprocal transmission tests, ELISA may be used to determine transmission efficiency of various thrips vector species on different crop plants. Future testing will determine the utility of these data for development of disease prediction models.

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